REMARKS

Claims 1, 3, 4, 7-18, 20, and 21 are currently pending in the application. Claim 21 has been withdrawn as drawn to non-elected subject matter. By the present communication, claims 1, 9, 12 and 17 are amended and claim 22 is canceled without prejudice or disclaimer. The amended claim language is supported throughout the as-filed specification, the Examples, and the claims as originally filed. No new matter has been added. Applicants note that claim 1 has been amended to recite the limitation of canceled claim 22 and claims 9, 12, and 17 have been amended to clarify grammar. After entry of the present amendment, claims 1, 3, 4, 7-18, 20, and 21 will be under consideration.

Claims Objections

Claims 9, 12 and 22 have been objected for allegedly having various informalities. Without acquiescing to the allegations presented in the Office Action, Applicants have amended claims 9 and 12 rendering the objections moot. Specifically claim 9 has been amended to depend from claim 1; and claim 12 has been amended to recite "the method". Additionally, claim 22 has been canceled rendering the objection moot. Accordingly, Applicants respectfully request withdrawal of the objections.

Rejection Under 35 U.S.C. §112, Second Paragraph

Applicants traverse the rejection of claims 17-18 and 20 under 35 U.S.C. §112, second paragraph, as allegedly being indefinite.

The Examiner asserts that recitation of "the IGF2 gene" in claim 17 fails to have proper antecedent basis. Without acquiescing to the allegations presented in the Office Action, Applicants have amended claim 17 to recite "a DMR of an IGF2 gene". As amended, the proper antecedent basis is provided for recital of "the IGF2 gene" in lines 4-6 of the claim.

Accordingly, reconsideration and withdrawal of this rejection are respectfully requested.

Rejection Under 35 U.S.C. §112, First Paragraph, Written Description

Applicants traverse the rejection of claims 1, 3-4, 7-18, 20 and 22 under 35 U.S.C. §112, first paragraph as allegedly failing to comply with the written description requirement.

The Examiner asserts that recital of "hypomethylation is as compared to the half-methylation of the normally imprinted gene" is not supported by the specification. While acknowledging that the specification discloses hypomethylation as compared to half-methylation, the Examiner alleges that the specification fails to disclose the concept of "half-methylation of the normally imprinted gene", e.g., fails to disclose or define a "normally imprinted gene" as being a half-methylated gene. Applicants respectfully disagree.

In contrast to the position of the Examiner, the application clearly discloses a normally imprinted gene as being half-methylated in several instances throughout the specification. For example, paragraph [0168] states as follows:

> It was next determined whether a method of the present invention can be performed using DNA rather than RNA. SEQ ID NO:1 provides a differentially methylated region (DMR) within IGF2 that shows hypomethylation in CRC with LOI (Cui H. et al., Cancer Res. 62, 6442-6446 (2002), incorporated herein in its entirety by reference). *In order to determine whether a hypomethylation defect occurs in PBL and colon of patients without known neoplasia, we examined 24 samples, 12 from normal tissues (6 PBL, 6 matched normal colonic mucosa) with normal imprinting, and 12 from normal tissues (6 PBL, 6 matched normal colonic mucosa) with LOI. In all 12 tissues with normal imprinting, IGF2 showed a normal pattern of half-methylation (Fig. 2A).* In contrast, in 11 of 12 samples from normal tissue with LOI, IGF2 showed hypomethylation of the IGF2 DMR; in the other sample, IGF2 showed partial methylation of both alleles but was nevertheless abnormal (Fig. 2B). The significance of hypomethylation between normal tissues with and without LOI was $p < 0.0001$ (Fisher's exact test). In contrast, H19 showed hypomethylation in all cases, regardless of imprinting status (data not shown). Thus, aberrant IGF2 methylation is linked to LOI in normal colon and lymphocytes, just as it is in CRC. (emphasis added)

Paragraph [0168] states that 24 samples were examined, 12 from normal tissues with normal imprinting. With regard to these 12 samples, paragraph [0168] further states that "[I]n all 12 tissues with normal imprinting, IFG2 showed a normal pattern of half-methylation."

Again in paragraph [0178], samples with normal imprinting are detailed as having a normal half-methylation pattern at the *IGF2* DMR. For example, paragraph [0168] states as follows:

> Hypomethylation of *H19* and *IGF2* DMRs in Primary CRCs. To determine whether hypomethylation was also linked to LOI in primary colon cancers, we then analyzed 20 CRCs informative for imprinting status of *IGF2* (heterozygous for a transcribed polymorphism) by reverse transcription-PCR, 12 with LOI *and 8 with normal imprinting. All 8 of the CRCs with normal imprinting showed the normal half-methylation pattern at the IGF2 DMR*, and all 12 of the CRCs with LOI showed marked hypomethylation of the *IGF2* DMR ($P = 0.000007$; Figs. 5 and 6). In tumors with normal imprinting, the fraction of CpG sites that were methylated was $43.6 \pm 10.9\%$, whereas in tumors with LOI the fraction of sites methylated was $10.9 \pm 9.4\%$ ($P < 0.0001$). In addition, for each DMR, 15–20 clones were independently sequenced from the PCR product of each bisulfite-treated sample, and each experiment was repeated at least once. We also observed hypomethylation of the *H19* DMR in CRC, although the differences were not absolute as in the case of the *IGF2* DMR, but were in marked contrast to Wilms' tumors with LOI (Table 4). These results also differ markedly from those of Nakagawa et al. (H. Nakagawa et al, Proc. Natl. Acad. Sci. USA, 98: 591-596, 2001), who reported hypermethylation of CBS6 in colorectal cancer. Finally, because LOI is found at increased frequency in both tumor and normal tissue of patients with CRC, we also examined the matched normal mucosa of 3 CRC patients whose tumors showed LOI. As we reported earlier (H. Cui et al, Nat. Med., 4: 1276-280, 1998), the matched normal mucosa also showed LOI of *IGF2*, although methylation had not been examined in that study. We found the same pattern of hypomethylation in the normal colonic mucosa in each patient as we found in tumors (Table 4), indicating that this epigenetic abnormality was not limited to the cancers.

Paragraph [0178] states that 8 samples exhibited normal imprinting. With regard to these 8 samples, paragraph [0178] further states that "[A]ll 8 of the CRCs with normal imprinting showed the normal half-methylation pattern at the *IGF2* DMR...." As such, the application clearly discloses a normally imprinted gene as being half-methylated.

Further, in paragraph [0181], samples with normal imprinting are again detailed as having a normal half-methylation pattern at the *IGF2* DMR. For example, paragraph [0181] states as follows:

> The second major result of this study provided in this Example is that normal imprinting in the colon and LOI in CRC is specifically linked to the methylation status of a DMR within *IGF2* and to a lesser extent to the methylation status of *H19*. *Thus, all 8 of the cancers with normal imprinting showed normal half-methylation of the IGF2 DMR* and all 11 of the cancers showed hypomethylation of this DMR, as well as 3 matched normal mucosal specimens that also showed LOI. Takai *et al.* (37) recently described partial or complete hypomethylation of the *H19* ICR in two of four bladder cancers, but no relationship to *H19* imprinting; *IGF2* was not examined in that study. No alteration of *H19* imprinting was observed in the CRC examined here. It has been reported earlier that cancers with LOI also show LOI in the matched normal mucosa (Cui, H., Horon, I.L., Ohlsson, R., Hamilton, S.R., Feinberg, A.P. Loss of imprinting in normal tissue of colorectal cancer patients with microsatellite instability. Nat. Med., 4: 1276-280, 1998), so we would expect that this methylation abnormality is generally present in the colon of these cancer patients, as disclosed in Example 1.

Based at least on the foregoing identified portions of the specification, Applicants submit that recital of "hypomethylation is as compared to the half-methylation of the normally imprinted gene" in the claims is fully supported by the specification. Accordingly, reconsideration and withdrawal of the rejection are respectfully requested.

Rejection Under 35 U.S.C. §112, First Paragraph, Enablement

Applicants traverse the rejection of claims 1, 3-4, 7-18, and 20 under 35 U.S.C. §112, first paragraph as allegedly not enabled by the specification..

The Examiner acknowledges that the specification is enabling for methods in which a biallelic absence of methylation at positions 87, 90, and 106 of SEQ ID NO:1 is detected in human blood or colonic mucosal samples as correlating with LOI of the IGF2 gene in human colorectal cancer (CRC) patients and as an indicator of CRC risk is subjects. However, the

Examiner asserts that the specification is not enabling for methods employing detection of any other type of hypomethylation within H19 and/or IGF2 genes as indicators of LOI of H19 and/or IGF2 or as indicators of cancer risk or for non-human subjects or other forms of cancer. The Examiner notes that the previous amendments to the claims have overcome portions of the rejection of record, and acknowledges enablement of the invention at least with respect to hypomethylation at particular sites (positions 87, 90, and 106 of SEQ ID NO:1) in specific sample types. Further the Examiner acknowledges that the invention is enabled at least with regard to the particular sample types recited in claim 22.

Without acquiescing to the rationale presented in the Office Action, Applicants have amended claim 1 to recite the particular sample types recited in canceled claim 22. As such, claim 1 recites that the biological sample is a blood sample or a colon mucosa sample.

With regard to the Examiner's assertion that the invention lacks enablement for hypomethylation at positions other than position 87, 90, and 106 of SEQ ID NO:1 being linked to the LOI of IGF2 in colorectal cancer, Applicants respectfully disagree. Applicants assert that the specification clearly enables one skilled in the art to practice the invention commensurate in scope with the claims as amended. Claim 1, is directed to a method for identifying loss of imprinting (LOI) of the IGF2 gene in a subject with colorectal cancer, by analyzing a biological sample, a blood sample or colonic mucosal sample, from the subject for hypomethylation of a differentially methylated region (DMR) of at least one of the H19 gene and the IGF2 gene and detecting hypomethylation of the DMR in the subject, wherein hypomethylation is as compared to the half-methylation of the normally imprinted gene, and wherein further the DMR of the IGF2 gene comprises SEQ ID NO:1, wherein detection of hypomethylation of the DMR in the subject correlates with loss of imprinting (LOI).

The specification clearly discloses that hypomethylation, rather than hypermethylation, is linked to the LOI of IGF2 in colorectal cancer. For example, paragraph [0180] states as follows:

> First, hypomethylation, rather than hypermethylation, is linked to LOI of *IGF2* in human CRC based on two lines of evidence. In CRC lines in which hypomethylation is induced artificially by *DNMT1/DNMT3B* double knockout, LOI is found only in the hypomethylated lines. Indeed, unmodified HCT116 cells with hypermethylation of the *H19* DMR exhibit

> normal imprinting, even though Wilms' tumors with hypermethylation of the same sites show LOI (27). Furthermore, in primary human CRC, as well, LOI is linked to hypomethylation rather than hypermethylation. The latter result is in contrast to the findings of Nakagawa *et al.* (35), who reported hypermethylation of the *H19* in CRC with LOI of *IGF2*.

As previously pointed out by the Applicants, with regard to the latter line of evidence mentioned above, the present disclosure provides evidence that hypomethylation, that is, decreased methylation relative to the half-methylation status of the normally imprinted gene, of a DMR in IGF2 is associated with CRC. In Example 2, three exemplary CpG sites of a DMR upstream of exon 3 in the IGF2 gene (i.e., positions 87, 90, and 106 of SEQ ID NO:1) were examined for methylation status. The results in Table 4 indicate that of 20 CRCs informative for imprinting status of IGF2, the 12 samples with LOI all showed marked hypomethylation of the IGF2 DMR, whereas the 8 samples with normal imprinting showed the normal half-methylation pattern at the IGF2 DMR. These results are consistent with early reports describing "the first epigenetic alterations found in human cancer was hypomethylation of DNA and that CRC show global hypomethylation even in the presence of specific sites of increased DNA methylation" (specification at paragraph [0180]). Thus, it appears that it is the overall hypomethylation of this DMR, relative to the half-methylation normally imprinted gene, that is important, rather than the specific sites which are hypomethylated. Indeed, a recent report by Murrell *et al.* (PLOS One 3(3):e1849, 2008; copy attached; hereinafter "Murrell") supports these findings. In particular, the authors evaluated the same IGF2 DMR (termed "DMR0"), including the same 3 CpG, as well as 3 others within this DMR, and found that hypomethylation was associated with LOI of IGF2.

Regarding Murrell, the Examiner assert that an application must be enabling with respect to a claimed invention as of the time the invention was made. The Examiner notes that Murrell was published several years after the filing date of the present application and therefore improperly concludes that the reference cannot be relied upon with regard to the state of the art or the enablement of the invention.

However, Applicants assert that it is well established that Applicants are not precluded from "providing a declaration after the filing date which demonstrates that the claimed invention

works." M.P.E.P. § 2164.05. "While a later dated publication cannot supplement an insufficient disclosure in a prior dated application to make it enabling, applicant can offer the testimony of an expert based on the publication as evidence of the level of skill in the art at the time the application was filed." M.P.E.P. § 2164.05(b) (*citing Gould v. Quigg*, 822 F.2d 1074, 1077, 3 USPQ2d 1302, 1304 (Fed. Cir. 1987)). Similarly, later dated publications may properly be submitted as evidence that a disclosure was in fact enabled when it was filed. *See In re Brana*, 51 F.3d 1560, 1567, 34 USPQ2d 1436 n.19 (Fed. Cir. 1995). As stated by the Board of Patent Appeals and Interferences, "post-filing evidence can be relied on for certain purposes," one of which is "as evidence that the disclosed device would have been operative." *Ex parte Lal*, Appeal 2007-2517 (Bd. Pat.App. & Interf. 2007); *see also Ex parte Olson*, Appeal 2007-4153 (Bd. Pat.App. & Interf. 2008). This has been supported by the Federal Circuit in *Amgen Inc. v. Hoechst Marion Roussel,* 314 F.3d 1313, 1336 (Fed. Cir. 2003) where the court allowed use of post-filing publications to demonstrate enablement.

As such, it is proper for Applicants to rely on Murrell as evidence of enablement of the claimed invention. Based on the foregoing, as well as arguments previously submitted on the record. Applicants submit that the amended claims are fully enabled by the specification. Accordingly, reconsideration and withdrawal of the rejection are respectfully requested.

CONCLUSION

Applicants submit that the pending claims are in condition for allowance. The Examiner is invited to contact Applicants' undersigned representative if there are any questions relating to this submission.

Commissioner is hereby authorized to charge the total amount of $960.00 to cover the payment of a Request for Continued Examination fee ($405.00) and a Three-Month Extension of Time fee ($555.00), small entity, to Deposit Account No. 07-1896. The Commissioner is further authorized to charge any additional fees, or make any credits, to Deposit Account No. 07-1896 referencing the above-identified attorney docket number.

Respectfully submitted,

Date: August 31, 2010

Lisa A. Haile, J.D., Ph.D.
Registration No. 38,347
Telephone: (858) 677-1456
Facsimile: (858) 677-1465

DLA Piper LLP (US)
4365 Executive Drive, Suite 1100
San Diego, California 92121-2133
USPTO Customer Number 28213